Rotation thromboelastometry (ROTEM®) stability and reproducibility over time

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Abstract: Background: Thromboelastometry is a whole blood assay performed to evaluate the viscoelastic properties during blood clot formation and lysis. Rotation thromboelastography (ROTEM((R)), Pentapharm GmbH, Munich, Germany) has overcome some of the limitations of classic thromboelastography. So far, no clinical validation on reproducibility (inter- and intra-assay variability) and sample stability over time has been published. Methods: To evaluate the pre-analytic aspects, sample stability over time was assessed in 48 patients in eight age groups. Citrated blood was stored at room temperature. Tests measured every 30min from T 0min up to T 120min on two ROTEM((R)) devices were INTEM (ellagic acid activated intrinsic pathway), EXTEM (tissue factor-triggered extrinsic pathway) and FIBTEM (with platelet inhibitor (cytochalasin D) evaluating the contribution of fibrinogen to clot formation). Precision by intra- and inter-assay variability was evaluated at two points of time in 10 volunteers. Finally, reference intervals and effect of age and sex were evaluated. Results: Blood was stable over 120min and no significant differences in ROTEM((R)) results were found. Maximum clot firmness measurements had a coefficient of variation of <3% for EXTEM, <5% for INTEM and <6% for FIBTEM. For clot formation time, the coefficient of variation was <4% for EXTEM and <3% for INTEM. Coefficient of variation for angle alpha was <3% for EXTEM and <6% for INTEM. The coefficient of variation for clotting time was <15% for both EXTEM and INTEM. Small but significant differences between ROTEM((R)) devices were found for maximum clot firmness in FIBTEM and INTEM as well as clot formation time and alpha angle in INTEM. Conclusions: ROTEM((R)) yields stable results over 120min with a minimal variability on the same ROTEM((R)) device. However, small but significant differences between ROTEM((R)) devices were observed. Analysis should be performed on the same ROTEM((R)) device if small differences are of importance for treatment.

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Rotation thromboelastometry (ROTEM®) stability and reproducibility over time☆

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Abstract

Background: Thromboelastometry is a whole blood assay performed to evaluate the viscoelastic properties during blood clot formation and lysis. Rotation thromboelastography (ROTEM®, Pentapharm GmbH, Munich, Germany) has overcome some of the limitations of classic thromboelastography. So far, no clinical validation on reproducibility (inter- and intra-assay variability) and sample stability over time has been published. **Methods:** To evaluate the pre-analytic aspects, sample stability over time was assessed in 48 patients in eight age groups. Citrated blood was stored at room temperature. Tests measured every 30 min from T 0 min up to T 120 min on two ROTEM® devices were INTEM (ellagic acid activated intrinsic pathway), EXTEM (tissue factor-triggered extrinsic pathway) and FIBTEM (with platelet inhibitor (cytochalasin D) evaluating the contribution of fibrinogen to clot formation). Precision by intra- and inter-assay variability was evaluated at two points of time in 10 volunteers. Finally, reference intervals and effect of age and sex were evaluated. **Results:** Blood was stable over 120 min and no significant differences in ROTEM® results were found. Maximum clot firmness measurements had a coefficient of variation of <3% for EXTEM, <5% for INTEM and <6% for FIBTEM. For clot formation time, the coefficient of variation was <4% for EXTEM and <3% for INTEM. Coefficient of variation for angle alpha was <3% for EXTEM and <6% for INTEM. The coefficient of variation for clotting time was <15% for both EXTEM and INTEM. Small but significant differences between ROTEM® devices were found for maximum clot firmness in FIBTEM and INTEM as well as clot formation time and alpha angle in INTEM. **Conclusions:** ROTEM® yields stable results over 120 min with a minimal variability on the same ROTEM® device. However, small but significant differences between ROTEM® devices were observed. Analysis should be performed on the same ROTEM® device if small differences are of importance for treatment.

Keywords: Thromboelastometry; Blood coagulation; Haemostasis; Thromboelastography

1. Introduction

Rotation thromboelastometry (ROTEM®) (ROTEM® delta, Pentapharm GmbH, Munich, Germany), a methodology based on thromboelastography originally described by Hartert more than 50 years ago [1], is frequently used today to rapidly assess the visco-elastic properties of the developing clot in cardiac and transplant surgery as well as following trauma [2–5]. ROTEM® documents the interaction of platelets with the coagulation factors from initial platelet–fibrin interaction, through platelet aggregation, clot strengthening and fibrin cross-linking to eventual clot lysis. Within 30 min, a ROTEM® tracing provides information on clotting factor activity, platelet function and any clinically significant fibrinolysis [6,7].

The goal of this study was to assess stability and reproducibility over time of ROTEM®. The lack of such a study was already mentioned by Dunning et al. [8] in 2008 and by Samama and Ozier [9] in 2003. Possible changes due to age and sex were also of interest. In thromboelastography (TEG® Haemoscope Corporation, Skokie, IL, USA), stability of results over time is only achieved following a 30-min waiting time after blood draw and before analysis [10]. Immediate sample analysis, however, is desirable in the setting of an acute bleeding event. We thus assessed stability over time for ROTEM®.

2. Material and methods

This clinical trial was performed after obtaining authorisation by the local ethics committee (Kantonale

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Ethikkommission, Kanton Zurich, Switzerland, Study number StV 27-2007).

Sample size ($n = 48$) was chosen based on statistical considerations and on reported general practice. Horn and Pesce showed that a minimum of 39 patients is needed for establishing a 95% reference interval by power analysis [11]. In addition, Friedberg et al. reported in 2007 [12], that 50% of laboratories enrol 21–50 subjects when establishing reference intervals. As a possible age or gender effect was of interest, three male and three female patients in each of the following eight age categories were included: below 20 years; 20–30 years; 30–40 years; 40–50 years; 50–60 years; 60–70 years; 70–80 years; and above 80 years.

Inclusion criteria were scheduled for a non-emergent operation and signed written informed consent. Exclusion criteria were known malignancy or immunosupression, known coagulation disorders, anticoagulation in any form, current treatment with heparin (other than routine preoperative thromboembolic prophylaxis with 3000 IU low-molecular-weight heparin administered subcutaneously the evening prior to the operation), use of acetyl salicylic acid within the past 5 days, use of non-steroidal anti-inflammatory agents within the past 24 h, known renal diseases or plasma concentration of creatinine more than 120 mM and liver diseases or increased plasma concentration of aspartate aminotransferase (>50 U l^{-1}) or alanine aminotransferase (>50 U l^{-1}) as well as patients not capable of understanding the German language.

The study's main objectives were to validate ROTEM® by investigating (1) pre-analytic aspects (sample stability), (2) reproducibility and precision of ROTEM® (intra- and inter-assay variability), and (3) reference intervals and effect of age and sex.

Several predefined tests were assessed: INTEM (ellagic acid activated intrinsic pathway), EXTEM (tissue factor triggered extrinsic pathway) and FIBTEM (with platelet inhibitor (cytochalasin D) evaluating the contribution of fibrinogen to clot formation). These three assays are performed in citrated samples and represent counterparts of routine tests of plasmatic coagulation: fibrinogen, prothrombin time and activated partial thromboplastin time. The two main differences between thromboelastometric tests and plasmatic coagulation tests are (i) the former is performed on whole blood, the latter on plasma, and (ii) the former measures processes involving thrombin generation, clot formation and clot lysis, the latter processes leading up and until the initial generation of thrombin. By using inhibitors of platelet function, the developers of the assay have derived a functional test of fibrinogen, called FIBTEM. This can be correlated with functional fibrinogen tests in plasma. By comparing EXTEM, the thromboelastometric test that uses tissue factor (comparable to the prothrombin time test) as an activator, with FIBTEM, one can deduce relevant information regarding platelet function and factor XIII function, as both of these contribute to EXTEM measurement.

In EXTEM, the extrinsic pathway is activated by thromboplastin from rabbit brain to assess clot formation and fibrinolysis. In INTEM, the intrinsic pathway is activated by a contact activator to assess the clot formation and fibrin polymerisation. In FIBTEM, the extrinsic pathway is activated by tissue factor in presence of a platelet inhibitor to assess the functional fibrinogen level.

Ten volunteers were sufficient to investigate precision and reproducibility according to power analysis with 95% confidence interval (CI). To investigate precision and reproducibility of ROTEM® analysis, for inter-assay (on one single ROTEM® device), reproducibility testing was performed in the volunteers, after having obtained written informed consent, withdrawing three tubes (Vacutainer Brand, Belliver Industrial Estate, Plymouth, UK, 4.5 ml, 9 NC 0.129 M, a total of 18 ml per volunteer) of citrated blood, the first tube (Vacutainer Brand, Belliver Industrial Estate, Plymouth, UK, 4.5 ml, 9 NC 0.129 M) drawn was discarded to exclude coagulation activation due to vein puncture and blood withdrawal. Inter-device variability was tested using the same samples on a second ROTEM® device. Intra-assay variability was tested by performing duplicate measurements at the time of the first blood withdrawal on the same device using different channels and calculating the coefficient of variation. At a second point of time, that is, 1 week later, another three tubes (Vacutainer Brand, Belliver Industrial Estate, Plymouth, UK, 4.5 ml, 9 NC 0.129 M, a total of 18 ml per volunteer) of citrated blood were again withdrawn from the same 10 volunteers to assess the reproducibility of the two ROTEM® devices at the second point of time (week 2) as compared with the first week. The 10 volunteers were nurses or physicians from the Institute of Anaesthesiology, of the University Hospital of Zurich, and the tests ran for 30 min.

After obtaining written informed consent from the patients, eight tubes (Vacutainer Brand, Belliver Industrial Estate, Plymouth, UK, 4.5 ml, 9 NC 0.129 M) of citrated blood were withdrawn, a total of 36 ml of blood per patient. The first tube (Vacutainer Brand, Belliver Industrial Estate, Plymouth, UK, 4.5 ml, 9 NC 0.129 M) drawn was discarded to exclude coagulation activation due to vein puncture and blood withdrawal. In the 48 patients, INTEM, EXTEM and FIBTEM were performed at T 1 = 0 min, T 2 = 30 min, T 3 = 60 min, T 4 = 90 min and T 5 = 120 min. The blood was stored at room temperature. Tests were performed at 37 °C. Measurements for this test series ran for 60 min.

2.1. Parameters of rotation thromboelastometry

ROTEM® defines various parameters to describe the dynamics, the size and the firmness of clot during clot formation and lysis (Fig. 1). The clotting time (CT) is the period from the start of the analysis until the start of clot formation, normally until the 2-mm amplitude is reached. The clot formation time (CFT) is defined as the period until an amplitude of 20 mm is reached. The angle alpha is given by the angle between the centre line and a tangent to the curve through the 2-mm amplitude point. The maximum amplitude of the curve is defined as the maximum clot firmness (MCF). The amplitude at different points of time is described by A5 till A30, whereby the number refers to the time since the start of the test. The clot lysis index (CLI) at 30 and 60 min (CLI30, CLI60) describes the ratio between the maximum clot firmness and the amplitude 30 and 60 min after clotting time, and gives information about the fibrinolysis. The maximum lysis (ML) represents the maximum fibrinolysis detected during the measurement.

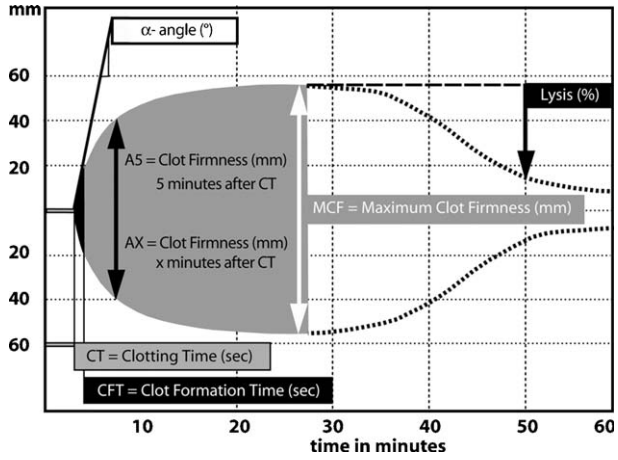

Fig. 1. Modified, with authorisation of Pentapharm GmbH, Munich, Germany. CT (clotting time): time from the start of measurement until initiation of clotting – initiation of clotting, thrombin formation, start of clot polymerisation. CFT (clot formation time): time from initiation of clotting until a clot firmness of 20 mm is detected – fibrinpolymerisation, stabilisation of the clot with thrombocytes and F XIII. MCF (maximum clot firmness): firmness of the clot – increased stability of the clot by the polymerised fibrin, thrombocytes as well as F XIII. Lysis: reduction of the clot firmness after MCF in relation to MCF – stability of the clot. A maximum lysis (ML) <15% is considered normal, a ML >15% within 1 h is indicative of exaggerated fibrinolysis (hyperfibrinolysis).

The parameters measured in this study were: clotting time, clot formation time, maximum clot firmness, angle alpha, and amplitude after 5, 10, 15, 20, 25 and 30 min.

2.2. Test procedure

All ROTEM® devices used in this study were new and set up by a representative of the local distributor. The tests were performed using the automated pipette programmes according to the instructions of the manufacturer. For each measurement, a new pin was positioned on the axis of the measurement channel and a new cup was put into the special cup holder of the device. According to the pipetting programme, 20 μl re-calcification reagent (200 mmol l^{-1} calcium chloride solution) and 20 μl of the respective activation reagent (FIBTEM: cytochalasin D, EXTEM: thromboplastin from rabbit brain and INTEM: partial thromboplastin phospholipid made of rabbit brain (chloroform extract), ellagic acid) were added into the pre-warmed cup. Then, 300 μl of citrated whole blood was added to the cup and, after a semi-automated mixing step, the cup holder was placed in the measuring position of the ROTEM® device. The measurement started automatically when blood was added to the cup and was stopped after 30 or 60 min according to the protocol (EXTEM lot 41194401, INTEM lot 41166301, FIBTEM lot 41147601, star-TEM lot 41166101).

2.3. Statistical analyses

A trial database within Excel (Microsoft Office 2003, Microsoft Corporation Redmond, WA, USA) was used to store study data (transferred from the ROTEM® devices). The statistical analyses were performed using SPSS® (version 13, SPSS Inc., Chicago, IL, USA). Continuous variables are summarised as mean ± SD and median with CI where appropriate. Analysis of variance (ANOVA) for repeated measures was used to analyse all parameters with *post hoc* comparison and Bonferroni correction. For age and gender, regression analyses were used. *p* Values of 0.05 or less are considered significant.

3. Results

3.1. Baseline information

The 48 patients had a mean age of 50 ± 22 years, ranging from 17 to 87 years. For the 24 women, the mean age was 50 ± 22 years, ranging from 17 to 85 years. For the 24 men, the mean age was 50 ± 22 years, ranging from 17 to 87 years. The 10 volunteers were five men and five women; the overall mean age was 37 ± 14 years, ranging from 20 to 63 years.

3.2. Pre-analytic aspects' stability over time

Over the time of 120 min, there was no significant difference between any parameter in EXTEM, INTEM and FIBTEM, indicating that ROTEM® measurements are stable over 2 h at room temperature (Table 1).

3.3. Reproducibility and precision of ROTEM® devices (intra- and inter-assay variability)

The reproducibility of results on two ROTEM® devices was tested with 10 volunteers. Tests were performed twice with a week's interval in between; and an overall analysis was performed between the two points of time (week 1 vs week 2) and the two ROTEM® devices.

The reproducibility of maximum clot firmness in the 10 volunteers at the two points of time in the two ROTEM® devices showed no significant difference between the two points of time ($p > 0.200$) and there was no influence by the ROTEM® device ($p > 0.200$) in the overall effect for EXTEM (mean 63.0 ± 5.5 mm, reference range 53–72 mm, mean difference −0.1 mm, 95% CI −0.8 to 0.7 mm, maximum difference 1.3%).

For the maximum clot firmness of FIBTEM (mean 11.9 ± 3.7 mm, reference range 9–25 mm, mean difference 0.6 mm, 95% CI 0.2–1.0 mm, maximum difference 8.4%) and INTEM (mean 61.6 ± 4.3 mm, reference range 53–72 mm, mean difference 1.7 mm, 95% CI 1.2–2.2 mm, maximum difference 3.6%), a significant difference between devices (FIBTEM $p = 0.005$, INTEM $p < 0.001$) but not for the points of time (FIBTEM $p > 0.200$, INTEM $p = 0.187$) was found in the overall analysis (Table 2, Fig. 2).

For EXTEM and FIBTEM, no significant difference between devices or time points was found for clot formation time, clotting time and angle alpha (Table 2). The clot formation time ($p = 0.001$, mean 74.5 ± 20.1 s, reference range 35–110 s, mean difference −7.1 s, 95% CI −10.4 to −3.7 s, maximum difference 14.0%) and angle alpha ($p = 0.013$, mean 75.2 ± 3.7°, reference range 70–83°, mean difference 1.2°, 95% CI 0.3–2.1°, maximum difference 2.8%) were significantly different between devices in INTEM, but no difference for the two points of time was found. For the two

Table 1
Sample stability over the time, mean values and standard deviation.

	CT (s)					CFT (s)				
	T 0	T 30 min	T 60 min	T 90 min	T 120 min	T 0	T 30 min	T 60 min	T 90 min	T 120 min
INTEM										
Mean ± SD	152.3 ± 20.6	153. 6 ± 16.9	154.7 ± 20.0	156.5 ± 21.4	156.0 ± 20.1	64.7 ± 15.1	62.9 ± 15.4	64.1 ± 16.1	61.3 ± 15.5	61.6 ± 14.8
EXTEM										
Mean ± SD	55.3 ± 6.4	56.0 ± 6.1	56.9 ± 7.3	56.0 ± 6.3	55.0 ± 6.4	80.3 ± 20.7	79.0 ± 18.6	80.1 ± 21.4	77.3 ± 20.0	77.9 ± 19.2
FIBTEM										
Mean ± SD	53.5 ± 7.7	52.9 ± 5.4	53.0 ± 6.9	52.2 ± 5.6	53.7 ± 11.4	585.6 ± 553.9	696.7 ± 900.2	592.4 ± 847.2	506.4 ± 457.8	817.6 ± 941.7

	Alpha (°)					MCF (mm)				
	T 0	T 30 min	T 60 min	T 90 min	T 120 min	T 0	T 30 min	T 60 min	T 90 min	T 120 min
INTEM										
Mean ± SD	77.1 ± 2.8	77.5 ± 2.9	77.2 ± 2.9	77.7 ± 2.8	77.6 ± 2.9	63.5 ± 5.2	64.5 ± 5.0	63.8 ± 5.2	64.4 ± 5.1	64.0 ± 5.1
EXTEM										
Mean ± SD	73.9 ± 4.1	74.0 ± 3.8	74.0 ± 4.2	74.4 ± 3.9	74.2 ± 3.8	66.7 ± 5.5	66.3 ± 5.0	66.2 ± 5.3	66.3 ± 5.1	66.5 ± 5.3
FIBTEM										
Mean ± SD	69.9 ± 6.9	71.2 ± 5.3	70.8 ± 6.3	70.1 ± 6.1	71.8 ± 5.0	16.1 ± 5.9	16.3 ± 5.8	16.6 ± 6.0	16.7 ± 6.2	16.1 ± 5.6

MCF: maximum clot firmness, CFT; clot formation time, CT; clotting time.

Table 2
MCF: maximum clot firmness, CFT: clot formation time, CT: clotting time and angle alpha changes between two ROTEM® devices and weeks 1 and 2, mean values and standard deviations.

Test	Week	Device	*N*	MCF (mm) mean ± SD	CFT (s) mean ± SD	CT (s) mean ± SD	Angle alpha mean ± SD
EXTEM	1	1	10	63.3 ± 5.5	104.5 ± 29.1	56.7 ± 13.0	69.4 ± 5.4
EXTEM	1	2	10	63.1 ± 5.8	98.3 ± 31.1	61.0 ± 11.5	70.5 ± 5.8
EXTEM	2	1	10	62.7 ± 5.5	99.3 ± 24.6	59.0 ± 6.5	70.3 ± 4.7
EXTEM	2	2	10	62.8 ± 5.3	96.8 ± 25.1	58.9 ± 4.5	70.6 ± 4.9
FIBTEM	1	1	10	11.4 ± 4.0*	n.a.	57.9 ± 10.4	n.a.
FIBTEM	1	2	10	12.2 ± 4.2*	n.a.	57.3 ± 7.3	n.a.
FIBTEM	2	1	10	11.7 ± 3.2*	n.a.	54.6 ± 7.2	n.a.
FIBTEM	2	2	10	12.1 ± 3.2*	n.a.	54.1 ± 5.2	n.a.
INTEM	1	1	10	60.9 ± 4.8*	77.2 ± 22.5*	155.7 ± 17.7**	74.8 ± 4.0*
INTEM	1	2	10	62.9 ± 4.3*	68.8 ± 21.1*	157.0± 12.0**	76.1 ± 4.0*
INTEM	2	1	10	60.5 ± 4.0*	78.8± 18.6*	164.1 ± 12.6**	74.4 ± 3.4*
INTEM	2	2	10	61.9 ± 4.0*	73.1 ± 18.0*	160.3 ± 18.7**	75.5 ± 3.4*

* $p < 0.050$ for ROTEM® devices.
** $p < 0.050$ for the weeks.

points of time, a significant difference was only found for the clotting time (mean week one 156.4 ± 14.9 s, mean week two 162.2 ± 15.7 s, reference range 100–240 s) in INTEM ($p = 0.046$). For FIBTEM, the only analysis that is of importance is maximum clot firmness; all other values are not to be used in the interpretation of FIBTEM.

Reproducibility of results for maximum clot firmness was 97% in EXTEM, 95% in INTEM and 94% in FIBTEM. The coefficient of variation was calculated in the first point of time (week 1) on the same device. Coefficient of variation for maximum clot firmness in EXTEM was <3%, INTEM <5% and FIBTEM <6%. For clot formation time, the coefficient of variation was <4% for EXTEM and <3%. INTEM. Coefficient of variation for the angle alpha was <3% for EXTEM and <6% for INTEM. Clotting time had a coefficient of variation of <15% for EXTEM and INTEM.

3.4. Early prediction of maximum clot firmness

As tests for the 48 patients exceeded 60 min, we calculated for each point of time the percentage of the final maximum clot firmness, showing that in an overall analysis, after 10 min of running time, maximum clot firmness in EXTEM, INTEM and FIBTEM reached at least 98% of the final maximum clot firmness value.

3.5. Reference intervals

Reference intervals were calculated by means, according to international guidelines, and adding one standard deviation on either side. We also calculated reference intervals by gender (Table 3). We were able to show that reference intervals calculated in this study were closer together than those set by the manufacturer. A gender-specific influence is also to be seen.

3.6. Effect of age and gender

With advancing age, maximum clot firmness increased significantly in all tests ($p < 0.001$) by a mean of 0.1 mm per year of age over 20 years. Angle alpha also increased significantly for all tests with a mean of 0.07° per year of age

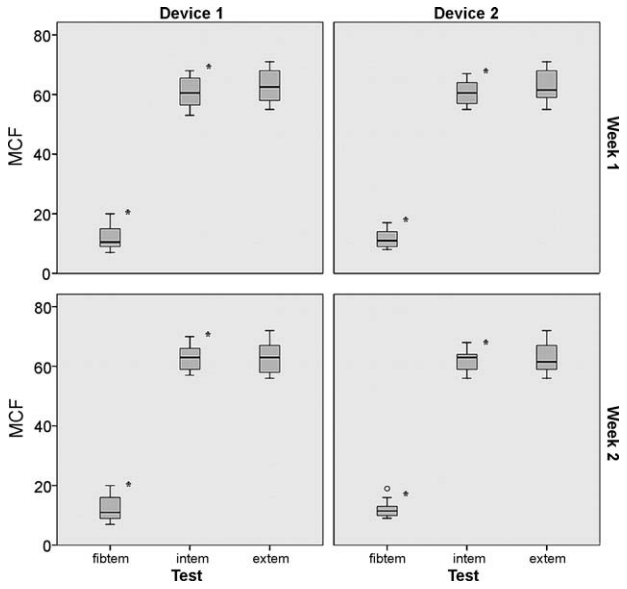

Fig. 2. Boxplot of MCF: maximum clot firmness for all tests, representing median by device 1 and 2 and point of time 1 and 2, *$p < 0.050$ for differences between ROTEM® devices.

Table 3
Reference values by the manufacture, reference values calculated in this study and calculated by gender in this study.

	Manufacturer	Overall	Male	Female
EXTEM MCF (mm)	50–70	61–72	59–72	64–72
INTEM MCF (mm)	50–72	59–68	56–68	61–69
FIBTEM MCF (mm)	9–25	10–22	8–22	13–21
EXTEM CFT (s)	34–159	61–97	62–108	59–86
INTEM CFT (s)	30–110	49–77	51–86	47–68
FIBTEM CFT (s)	n.a.	n.a.	n.a.	n.a.
EXTEM CT (s)	38–79	48–63	50–65	46–62
INTEM CT (s)	100–240	133–177	131–182	134–171
FIBTEM CT (s)	43–75	48–58	51–59	46–56
EXTEM angle alpha (°)	63–83	70–78	68–78	73–78
INTEM angle alpha (°)	70–83	75–80	73–80	76–81
FIBTEM angle alpha (°)	n.a.	n.a.	n.a.	n.a.

MCF: maximum clot firmness, CFT: clot formation time, CT: clotting time.

over 20 years in EXTEM ($p = 0.009$), 0.14° in FIBTEM ($p = 0.007$) and 0.04° in INTEM ($p = 0.019$). Clot formation time decreased significantly in EXTEM ($p = 0.008$) and INTEM ($p = 0.014$), with a mean of −0.4 s per year of age over 20 years in EXTEM and −0.2 s per year of age over 20 years in INTEM. The age had no influence on clotting time in all tests.

Maximum clot firmness ($p < 0.001$ for EXTEM and INTEM) and angle alpha ($p = 0.009$ in EXTEM, $p = 0.019$ in INTEM) were significantly higher and clot formation time lower ($p = 0.008$ in EXTEM, $p = 0.014$ in INTEM) in EXTEM and INTEM in women than in men, indicating a somewhat greater coagulability in women. Similar changes in FIBTEM (towards hypercoagulability) did not reach statistical significance. Clotting time was similar in all age groups and in both sexes (Table 4).

4. Discussion

Our analysis of pre-analytic aspects showed that ROTEM® tests yield stable results from citrated blood that was re-calcified during the first 120 min. This is of high practical relevance since the exact delay from blood drawing to testing would be difficult to standardise, particularly in major trauma. This is in contrast to the thromboelastography, where a previous report showed that parameters are unstable in the first 30 min after blood withdrawal [10].

Evaluation of the second aim (reproducibility: inter-assay, same device) showed a good reproducibility of results on the same ROTEM® device as well as over a time period of 120 min. Statistically significant differences on different ROTEM® devices occurred for the maximum clot firmness of FIBTEM and INTEM as well as the clot formation time and alpha angle of INTEM as mentioned above. For the interpretation of INTEM, these results are statistically significant but not of any clinical relevance because the values are largely within the reference values and would not be implemented in a treatment. Reference intervals calculated for our study and for gender were found to be within a narrower interval than those provided by the manufacturer. According to the manufacturer, the company's reference values are for orientation purposes and should be validated individually, as they may vary from lab to lab, depending on blood sampling technology and other pre-analytical factors.

Evaluation of the third aim (precision: inter-device) demonstrated reproducibility of ROTEM® measurements over time and when the blood sample is analysed on the same ROTEM® device sequentially. In addition, citrated blood samples analysed after 0–120 min yield similar results in all

Table 4
Sex related differences in the main parameters of ROTEM®, mean values and standard deviations.

	Age (years)	CT (s)	CFT (s)	Alpha angle (°)	MCF (mm)
INTEM					
Females ($n = 24$)	50.8 ± 21.9	152.5 ± 18.1	57.7 ± 10.5*	78.4 ± 2.1*	64.8 ± 3.8*
Males ($n = 24$)	52.0 ± 22.1	156.8 ± 25.5	68.1 ± 17.5*	76.5 ± 3.2*	62.2 ± 6.1*
EXTEM					
Females ($n = 24$)	50.8 ± 21.9	53.9 ± 7.8	72.6 ± 13.3*	75.3 ± 2.7*	68.1 ± 4.2*
Males ($n = 24$)	52.0 ± 22.1	57.7 ± 7.3	85.2 ± 23.2*	72.9 ± 4.6*	65.3 ± 6.4*
FIBTEM					
Females ($n = 24$)	50.8 ± 21.9	51.1 ± 5.0	n.d.	71.0 ± 5.6	17.3 ± 4.1
Males ($n = 24$)	52.0 ± 22.1	55.0 ± 4.2	n.d.	70.4 ± 6.5	15.0 ± 7.2

MCF: maximum clot firmness, CFT: clot formation time, CT: clotting time.
* $p < 0.050$ comparison male versus female.

ROTEM® tests assessed. However, when a blood sample is analysed on different ROTEM® devices simultaneously, statistically significant differences ($p < 0.050$) were detected in the overall comparison for the maximum clot firmness in FIBTEM (maximum difference 8.4%) and in INTEM (maximum difference 3.6%) as well as for clot formation time (maximum difference 14%) and angle alpha (maximum difference 2.8%) in INTEM. In clinical use, when using algorithms, a difference in maximum clot firmness of 1–2 mm in FIBTEM may result in a premature or delayed treatment of the patient. Therefore, when working with exact algorithms, the blood of one individual patient should be measured on one single ROTEM® device.

Compared with the results of Lang et al. [13], we have found a somewhat lower variability for the maximum clot firmness in the FIBTEM test (coefficient of variability for maximum clot firmness <6%). This may be due to the fact that, in our study, only three persons performed all ROTEM® tests, which is impossible when pooling data from different centres. Performing ROTEM® tests by persons familiar with the ROTEM® technology thus appear to reduce the variability.

A progressive change of the ROTEM® parameters towards hypercoagulability with advancing age as observed in this study has also been described by Ng [14] for thromboelastography (TEG® Haemoscope Corporation, Skokie, IL, USA), and is in keeping with previous reports describing hypercoagulability in elderly people [14,15]. Since the ROTEM® parameters measured in the current study were still within the reference ranges provided by the manufacturer, an age-specific adaptation of the reference ranges may not be required for ROTEM®. Nevertheless, the trend of increased clot firmness at advanced age should be kept in mind in the clinical interpretation of ROTEM® results.

We found differences between men and women in ROTEM® parameters, which indicate a faster development of the clot and greater clot strength in women as compared with men (Table 4). Unfortunately, no measurements of fibrinogen according to Clauss were performed in this study. Nevertheless, this may represent a factor predisposing women to thrombotic complications [16].

A limitation of this study is that we did not take into account the effective temperature in patients since it is known to impair results and interpretation of ROTEM® [17]. We think that this point is of limited importance since the ROTEM® devices work at a temperature of 37 °C and the 300 μl of blood drawn are at this same temperature and the patients were not hypothermic at the moment the blood was drawn.

ROTEM® results cannot give exact recommendations on the amount of blood products or coagulation factors to be administered. However, ROTEM® can guide the clinician as to which type of treatment may be most helpful to treat coagulopathy during surgery or in trauma. In 2007, Rugeri et al. demonstrated in trauma patients that ROTEM® can rapidly detect trauma-related coagulopathy and might be helpful in guiding treatment [18]. Theusinger et al. [19] published a ROTEM®-based transfusion algorithm to guide individual goal-directed transfusions. A retrospective analysis by Anderson et al. [20] in 990 patients showed that the use of ROTEM® significantly decreases the use of red blood cells and other blood products after cardiac surgery. This is particularly important given the fact that red blood cell transfusion in cardiac surgery is consistently associated with significant morbidity and increased mortality [21–24]. Last but not least, Spalding et al. have demonstrated that a ROTEM®-based coagulation algorithm decreased total transfusion costs in cardiac surgery [25].

5. Conclusion

ROTEM® measurements of EXTEM, INTEM and FIBTEM are reproducible and stable over time, regardless of delay from blood withdrawal to analysis (range 0–120 min after blood withdrawal). There is a high reproducibility with the coefficient of variation <6% in all assays. Coefficient of variation for maximum clot firmness in EXTEM was <3%, INTEM <5% and FIBTEM <6%; they are small but statistically significant. With age, there is a tendency of hypercoagulability and also with sex: women seem to coagulate better than men but within range. To avoid problems in treating coagulopathies when working with an algorithm or to interpret the evolution of ROTEM® parameters over time, ROTEM® tests should preferably be performed on the same ROTEM® device, because in our study one ROTEM® device yielded slightly but statistically significantly different results for maximum clot firmness and clot formation time.

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